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Review

Connexins and their environment: effects of lipids composition on ion channels

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Abstract

Intercellular communication is mediated through paired connexons that form an aqueous pore between two adjacent cells. These membrane proteins reside in the plasma membrane of their respective cells and their activity is modulated by the composition of the lipid bilayer. The effects of the bilayer on connexon structure and function may be direct or indirect, and may arise from specific binding events or the physicochemical properties of the bilayer. While the effects of the bilayer and its constituent lipids on gap junction activity have been described in the literature, the underlying mechanisms of the interaction of connexin with its lipidic microenvironment are not as well characterized. Given that the information regarding connexons is limited, in this review, the specific roles of lipids and the properties of the bilayer on membrane protein structure and function are described for other ion channels as well as for connexons.

Keywords: Connexin; Gap junction; Ion channel; Membrane lipid; Lipid–protein interaction; Cholesterol; Fatty acid; Lipid raft; Caveolin

Contents

1. Introduction

Gap junctions are complex, macromolecular structures that provide coordinated communication and signaling between neighboring cells. Intercellular communication between these specialized junctional assemblies is mediated through the family of connexin proteins. This family is encoded by ~20 different mammalian genes that are expressed in a wide variety of tissue types [1]. The connexin proteins (commonly designated as Cx*N*, with the value of *N* being the predicted MW in kDa) assemble as hexameric gap junction hemi-channels (termed connexons) that insert into the plasma membrane where they may tightly dock, end to end, with apposing hexamers in neighboring cells to provide

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an aqueous pore connecting the cell interiors [2,3]. As ion channels, these intrinsic membrane proteins coordinate cellular activity by allowing the controlled movement of ions, signaling molecules, and small metabolites (<1 kDa) between intracellular aqueous environments through the permeability barriers of adjacent cellular membranes [4]. These channels reside in the plasma membrane of virtually all metazoan cells and play a significant role in development, organ function, and disease processes [5–11]. Although gap junctions primarily function in intercellular communication, recent studies suggest that hemi-channels in the plasma membrane of some cells have important physiological and pathophysiological roles in diverse cellular activities such as inositol triphosphate-dependent Ca^{2+} wave propagation, ERK/MAPK signaling in anti-apoptotic protection, and ephaptic neuronal communication [12].

All of the members of the multigene connexin family share a common topology, with each subunit having four transmembrane domains, intracellular amino, and carboxy termini, one intracellular loop and two extracellular loops [13,14]. The two extracellular loops of the connexin subunits are highly conserved, allowing the requisite specific non-covalent contacts for connexon–connexon docking, with 2–3 nm gap between cells [15,16]. Initial models of the gap junction structure were based on X-ray diffraction studies [17] and electron microscopy studies [18] of the junctions in two-dimensional arrays. Crosslinking studies confirmed that the hemi-channel is hexameric [19]. A fairly high-resolution structure of a recombinantly over-expressed form of Cx43 without its carboxy-terminal domain has been determined by cryoelectron microscopy of two-dimensional crystalline arrays of the channel, and the three-dimensional density map confirmed that each of the six connexin subunits in a hemi-channel contains four transmembrane helices [20–22]. Given the high homology in the transmembrane (TM) domains of the connexin members, the Cx43 structure is considered paradigmatic for the entire family.

The extent of intercellular coupling is finely regulated by the control of the number of connexons present in the membrane, their functional state, and their permeability [1,23,24]. All of these properties are determined by the constituent proteins of the connexon and the interactions of these proteins with other protein partners and lipids. Connexin expression, cellular trafficking, and channel disassembly and degradation are relatively rapid processes that are finely controlled, with the connexin half-life generally being on the order of several hours [25]. The open state channel probabilities of the gap junction channels are regulated by pH, $[Ca^{2+}]$, membrane potential, the phosphorylation state of the constituent connexins, and other factors [26–28]. The roles of various kinases, phosphatases, cytoskeletal linking elements, and other proteins that interact with connexins as part of the multi-component pore complex have been recently reviewed [23,25,28–30]. Since the protein components of the multi-component complex may be enriched or depleted in lipid microdomains (described in more detail below), it follows that the lipid composition of the bilayer may play a key role in complex assembly and the modulation of connexon activity.

Given the shared environment of membrane proteins and the constituent lipids of the bilayer and the dynamic natures of both of these, their structures and functions alter and affect one another. In this review, we examine the effects of lipid composition on connexin function. The lipid components of the bilayer may act directly or indirectly on connexin function. These effects may be due to intermolecular binding events or via modulation of the bulk properties of the bilayer. Since the specific interactions between membrane constituents and connexin proteins are not as well resolved as for some other ion channels, we have also included in this survey examples of how lipid composition has been shown to affect other ion channels and membrane proteins [31,32]. Where pertinent, brief reviews of the physicochemical properties of lipid bilayers that may affect membrane protein structure and function are included for background.

2. Effects of the bilayer on membrane proteins

The lipid bilayer is a non-covalent association of amphipathic phospholipids, sphingolipids, glycolipids, sterols, and proteins in variable stoichiometries. The self-sealing assemblages that comprise the lipid bilayers of cells form spontaneously due to thermodynamic considerations driven by the hydrophobic effect. The bilayer provides a relatively impermeable structured environment between two aqueous compartments. It has diverse physicochemical properties that may vary widely in the planes normal and parallel to the bilayer surface. In general, between the two aqueous compartments on either side of the membrane, lipid bilayers have a central hydrophobic environment containing the acyl hydrocarbon chains of the lipid bilayer and a somewhat more complex interfacial region at either membrane surface that contains the polar lipid headgroups. This oversimplified view of the bilayer, schematized in Fig. 1, fails to represent the biophysical complexity of the bilayer since it may exist in several physical states.

The long hydrocarbon tails of these lipids are of variable length and saturation, with increased *cis* unsaturation, giving rise to kinks and decreased packing order. Differential packing as a function of bilayer composition gives rise to fairly complex phase diagrams for this packing. Bilayers that primarily contain phospholipids with saturated acyl chains exist in a solid–gel phase, L_β, characterized by restricted lateral motions of the tightly packed acyl chains. Enrichment with naturally occurring phospholipids with predominantly unsaturated acyl chains gives rise to the fluid liquid–crystalline phase, L_α, in which lipid packing is less

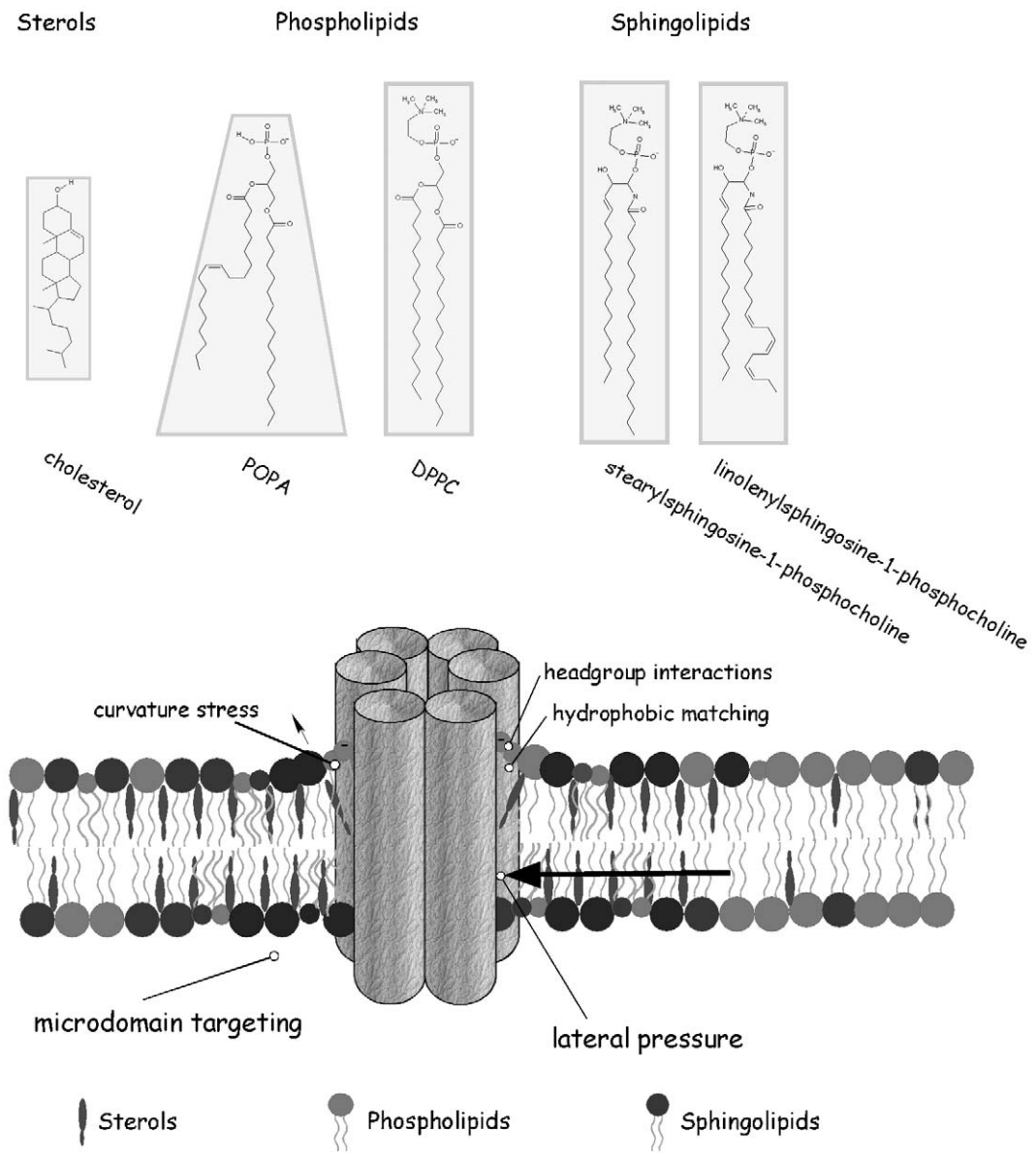

Fig. 1. Schematic drawing depicting some representative lipids and a cross section of a membrane bilayer containing a hexameric gap junction surrounded by lipid. Top panel gives the chemical representation of a typical sterol (cholesterol), phospholipids (palmitoyl oleoyl phosphatidic acid (POPA) and dipalmitoyl phosphatidylcholine (DPPC)), and sphingolipids (stearylsphingosine-1-phosphocholine and linolenylsphingosine-1-phosphocholine). The cross section of the bilayer in the bottom panel illustrates some of the physico–chemical properties of lipid bilayers that are discussed in the text.

ordered and there is a corresponding increased rate of lateral diffusion. Under physiological conditions, most membranes are generally considered to be in the fluid, liquid crystalline state. A more ordered liquid-ordered phase, L_o, is observed in mixtures containing enriched amounts of saturated phospholipids (such as those observed in sphingolipids) and cholesterol. Historically, these membranes were initially defined operationally by their insolubility to extraction by cold detergents, giving rise to their classification as lipid rafts [33]. These microdomains and their properties are further described in Section 3.3, "Connexons, lipid rafts, and caveolae". As described in this later section, differential segregation of membrane proteins to different lipid domains is considered to be critical in many cellular phenomena, including signaling and trafficking, and appears to be especially relevant to connexon function. Thus, membranes may exist in multiple states and undergo phase transitions as a function of temperature and lipid composition.

The polar headgroups of lipids line the interfacial region of the bilayer and have different volumes and chemistries that affect their interaction with other bilayer components and membrane proteins. Since lipids exhibit preferences for specific proteins and for each other, they may form microdomains such as the lipid shell around membrane proteins and lipid rafts that are enriched in specific subsets of lipids [34,35]. Furthermore, the inner and outer leaflets of

a membrane have different lipid compositions, and different cell types and organelles maintain different mixtures of lipids that are carefully modulated by the organism to optimize cellular activity. Overall, this rich diversity provides membrane-associated proteins with a variety of microenvironments with which to reside in and interact with.

The lipid components of the bilayer not only provide the framework in which membrane proteins reside, but they also specifically and non-specifically interact with membrane proteins affecting both their structure and function. The single most important energetic consideration driving the folding of TM domains of membrane proteins appears to be satisfying the H-bonding potential of peptide backbone amides and carbonyls [36]. Thus, TM domains often contain α-helices, which satisfy their H-bonding by intra-chain bonding, or sandwiches of β-strands, which have inter-strand H-bond networks. Lipid-exposed residues are typically very hydrophobic in nature, with a preference for aromatic amino acids, especially tryptophan and tyrosine, at the interfacial regions of the membrane surface [37–40]. Residues in the interior of membrane proteins are generally less hydrophobic than the residues in contact with acyl chains, and are comparable in hydrophobicity to residues in the hydrophobic interior of soluble proteins [41]. The determination of high-resolution structures for dozens of membrane proteins (a nice database of archived membrane protein structures may be accessed at http://www.blanco.biomol.uci.edu/Membrane_Proteins_xtal.html) has begun to elucidate the complex protein–protein and protein–lipid interactions that give rise to the architecture of these proteins [34]. Perhaps, the best understood protein–protein interactions in the membrane are those observed between TM α-helices [42,43]. In general, the specific interaction of helices is due to favorable van der Waals contacts in closely packed complementary surfaces, with Gly, Ala, and Ser residues being more likely to occur in the tight interfaces at helix crossing points [44]. Strongly polar amino acids are infrequently observed in the bilayer, but polar interactions between helices can also drive their oligomerization [45,46]. While the role of the complex hydrophilic/hydrophobic environment of the bilayer is critical for the folding of membrane proteins [36], specific lipids may bind to proteins and may be critical for protein activity. Analogous to the hydration shells observed in the crystal structures of proteins in an aqueous environment, some lipids can be resolved in the crystal structures of membrane proteins. A limited number of high-resolution structures of membrane proteins co-crystallized with lipids have provided evidence of specific lipid binding sites in the TM regions of the protein [47,48]. The TM regions of many membrane proteins of known structure have highly irregular surfaces presenting many grooves that are occupied with the acyl chains of the surrounding lipids. The driving force for these bound lipids is thought to arise from the energetics of the bilayer that favors a sealed surface (with respect to polar solvents such as water). Thus, adjacent lipids are under considerable strain to fill the irregular grooves. The preference for certain lipids at the protein–lipid interface may reflect how well these specific lipids fill the irregularities in the protein surface, as well as specific chemical interactions. Spin-label electron paramagnetic studies have shown that many integral membrane proteins retain a motionally-restricted annular monolayer of lipids about themselves [49]. Differential lipid affinities [50] and, in particular, the selective binding of a lipid shell by a membrane protein [35] may act to target that protein to particular lipid domains due to the compatibility of the bound lipid to selected microdomains (e.g., lipid rafts).

Given the thermodynamic constraints involved in passing through a biological membrane, it is also not surprising that most intrinsic membrane proteins, including ion channels, have an absolute requirement for lipid. In many cases, specific lipids are required for native structure and function [36,43]. The importance of these bound lipids to the function of some intrinsic membrane proteins is evident since the complete delipidation of solubilized membrane proteins often leads to irreversible denaturation. Recent successes in the crystallization of several membrane proteins in the presence of native lipids suggest an intimate involvement of individual lipid molecules with protein structure and stability. For example, electron diffraction studies of purple membranes indicated that the trimeric assembly of bacteriorhodopsin has many lipid-binding sites [51]. The importance of these interactions is reflected in a functional requirement for specific lipids in regulating the photocycle of this integral membrane protein [52]. In addition, membrane lipids are essential for KcsA refolding, and specific interactions with negatively charged lipid molecules are essential for ion conductance [53]. For a variety of ion channels, studies have indicated that particular combinations of lipids are often necessary for functional reconstitution [54]. Given these observations, it is expected that connexins have a similar requirement for specific lipids, but no information is currently available regarding this dependence.

Lipids may also act as molecular chaperones in the assisted folding of membrane proteins [55]. For example, phosphatidylethanolamine (PE) acts as a molecular chaperone for LacY [56], and lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, is necessary for the correct folding of OmpA [57] and PhoE channels [58]. Structural studies of OmpF pores [59], porin [60], and the FhuA transporter [61] have delineated specific binding sites for LPS. Some peptides or domains of proteins have been shown to adopt different conformations in aqueous solution or at a membrane surface. These amphitropic peptide–lipid interactions underlie the interactions of anti-microbial agents and peptide transduction domains with membranes of specific composition [62–64], as well as the selective infection by some

viruses [65]. In addition, the misfolding of oligomeric and fibrillar forms of the amyloidogenic peptides and proteins associated with Alzheimer's disease [66–70] and Parkinson's disease [71–73] on membrane surfaces shows a marked preference for distinct lipid components.

Thus, a picture emerges of a complex relationship between membrane proteins and the lipids of the bilayer. While the role of lipid composition on the structure and function of gap junctions has been investigated [24], the exact role of lipids remains poorly defined. In the following section, the current knowledge of the interaction of lipids with connexins is reviewed.

3. Connexin–lipid bilayer interactions

Defining the mechanistic role of a given lipid constituent on the function of gap junctions is difficult since any change in the composition of lipids within a membrane changes the multiple physical attributes of that membrane. Even the specific binding of lipids to a protein may be affected by the presence of other lipids since the membrane protein may be in competition with the lipid components of the membrane for binding to its preferred lipid. In addition, much of the membrane properties are mechanistically associated with the packing efficiency of bilayer components. While hydrogen bonding (e.g., the hydrogen bond between cholesterol and sphingomyelin) and electrostatic interactions (e.g., headgroup repulsion) occur between lipids, the major physical determinant that defines the physicochemical properties of the bilayer is related to how well the components of the membrane fit together.

3.1. Gap junction plaques and cholesterol

Analyses of the lipid composition of gap junction preparations isolated from various cell types and organisms after alkali or detergent extraction revealed a substantial enrichment in the relative cholesterol to phospholipid ratio [24], suggesting that cholesterol might play an important role in gap junction assembly. It was noted, however, that while the elevation in cholesterol is quite pronounced, it is difficult to compare the stoichiometries rigorously since the preparations were only examined after extraction, which may differentially alter the lipid composition. In addition, in some cell types, there was relatively high content of sphingolipids, as well as differential enrichment for some lipid headgroup moieties [74]. The role of cholesterol was further defined in studies in which gap junction assembly and permeability in cultured Novikoff hepatoma cells showed a dose-dependent response to supplemented exogenous cholesterol [75]. While the effective interactions of gap junctions with sterols have been known for a number of years, the exact nature of these interactions is not well defined.

In general, the plasma membranes of metazoans contain cholesterol at an elevated level of ~30–50 mol%. This important constituent of eukaryotic membranes has profound effects on the physical state of phospholipid bilayers. The tight enzymatic control of cholesterol transport and metabolism allows eukaryotic cells to modulate and refine the properties of their membranes by regulating their sterol content [76]. This small amphiphilic molecule contains a polar hydroxyl at the tip of its planar rigid steroid ring and a short hydrocarbon tail. Cholesterol partitions into bilayers such that its hydroxyl is in the interfacial region of the bilayer, approximately at the level of the ester carbonyl of the phospholipids, and the steroid ring and hydrophobic tail intercalate between adjacent acyl chains (for schematic, see Fig. 1). Cholesterol has been shown to affect membrane protein structure and function both by binding directly to specific binding sites on the protein and also by changing various biophysical properties of the bilayer [32]. The addition of cholesterol to a bilayer changes the fluidity, thickness, lateral pressure profiles, and the distribution of the surface charges of the bilayer. The role of fluidity in the cholesterol-rich environment of gap junctions was shown in studies in which heptanol was added to cardiac cells [77]. The cardiac gap junctions showed a decreased open probability that could be attributed to a reduction in the fluidity of its cholesterol-rich domains. In addition, oxidized cholesterol (specifically 7-ketocholestgerol) induces an increased communication through Cx43 in lens epithelial cells, presumably via an increase in Cx43 assembly and stability [78].

While there are many documented cases where the cholesterol and/or lipid composition affect membrane protein function, it has been especially difficult to exactly define the molecular mechanisms whereby these components affect the structure and function of ion channels and other membrane proteins. For example, while the absolute requirement for sterols and other specific lipids for the activity of nicotinic acetylcholine receptor (nAChR) has been known for decades [31], the specific mechanisms remain poorly defined and the subject of controversy. Neutral lipids, such as cholesterol, and anionic lipids, such as phosphatidic acid, are required to maintain receptor function during solubilization and reconstitution [79–82]. Delipidation studies in which the lipid/protein mole ratio fell below ~45 caused irreversible inactivation of the receptor, consistent with the requirement of an annular shell of lipids around the periphery of the hydrophobic region [83]. The nAChR exhibits a preference for certain lipids–in this case, neutral lipids and anionic phospholipids–but with a broad specificity [79,84–87]. This lack of specificity makes it difficult to distinguish between mechanisms that result from the direct binding of lipid to the receptor from those that result from changes in the biophysical properties of the membrane near the receptor. Neutral lipids and anionic phospholipids do not compete for their respective binding sites, offering an explanation as to why both types of lipids are needed for full ion channel activity [88–94]. The composition of the local lipid environment is thought to

modulate nAChR activity by shifting the complex equilibria between various states of the receptor (e.g., resting, open, or desensitized states).

In summary, the studies examining the role of cholesterol on connexon function suggest that cholesterol content plays a significant role in gap junctional activity. Some of these effects are further discussed in the context of lipid rafts below. However, the role of microdomain sequestration of gap junctions does not preclude specific, but as yet undiscovered, interactions of connexins with cholesterol. As noted by Malewicz et al. [24] in their review regarding lipid composition of purified plaques, earlier ultrastructural studies using cholesterol-binding compounds, such as filipin, to evaluate the cholesterol content of junctional regions suggested that the junctional areas had reduced cholesterol [95,96]. A retrospective evaluation of these initial studies suggests that the lack of available cholesterol for filipin binding may be due to cholesterol sequestration by the junctional protein, potentially due to its annular binding by connexin.

3.2. Lipid metabolites and connexon function

Phospholipids and sphingolipids are substrates for metabolic enzymes such as phospholipases, kinases, phosphatases, synthases, sphingomyelinases, and lipid transfer proteins [97,98]. Many of the resulting metabolites are signaling molecules and, when released, may act directly on their targets (e.g., binding as a specific ligand) or indirectly (e.g., by altering the properties of the bilayer). For example, phospholipases (PLA) selectively hydrolyze phospholipids releasing non-esterified fatty acids that regulate other membrane proteins [99,100]. Free fatty acids [101,102], as well as arachidonic acid and its metabolites [102], have been shown to uncouple neonatal cardiac gap junctions, suggesting a potential role of gap junctions in arrhythmia formation in ischemic heart disease. The molecular mechanism of this uncoupling, however, is unclear. Oleamides and arachidonamides, other endogenous fatty acid amides, have also been observed to inhibit gap junctional communication [103,104], perhaps via a similar uncoupling phenomenon [105]. Other examples of the effects of lipid metabolites on ion channels are the modulatory actions of free fatty acids on secretory chloride channels [106] and GABA receptors [107] and the direct activation of smooth muscle K^+ channels by arachidonic acid [108].

In osteocytes, the application of mechanical strain to osteocytes leads to altered function of their gap junctions [109]. The modulatory effect of this strain on connexon function may be direct and/or indirect. Typically, mechanosensory effects are mediated by changes in pressure. A consequence of packing different lipids species with each other and with proteins is the generation of lateral pressures in the membrane. This pressure may vary across the membrane and may affect protein conformation (for an excellent discussion, see Ref. [110]). Examples of membrane proteins unambiguously directly influenced by lateral pressure include the mechanosensitive channels [111–114]. In examining the major effect of shear stress on gap junctions in osteocyte membranes, the effects of stress were not mediated via lateral pressure, hydrophobic mismatch, or some other parameter of the membrane on connexin protein, but rather apparently due to its indirect effects. The primary effect on osteocyte gap junctions is via the conversion of arachidonic acid to prostaglandin E2 and a cascade of cellular events involving the prostaglandin receptor and activation of cAMP-dependent protein kinase (PKA), leading to increased expression and function of Cx43. Similar indirect effects have been shown to modulate other ion channels, most notably the family of K^+ channels. Lipid- and mechano-gated channels TRAAK and TREK are regulated by the properties of the bilayer in which they reside. These lipid-sensitive channels are sensitive to stretch, intracellular acidosis, temperature, anesthetics, and regulation by kinases and phosphatases. They are also reversibly opened by negatively charged polyunsaturated fatty acids such as arachidonic acid [113,115,116]. These channels are also activated by cone-shaped lysophospholipids (LPs) by an independent mechanism [117,118]. Similar dependence on lipid metabolites are exhibited by the weakly inward-rectifying ATP-sensitive potassium channels (K_{ATP}) that are regulated by membrane phosphatidylinositol phosphates that increase channel open probability and reduce sensitivity to ATP and other pharmacophores [119]. Inwardly rectifying channels (Kir) channels are also directly modulated by anionic phospholipids [120]. Many of the Kir channels directly bind PIP_2, with binding correlating with channel activity [121]. Analogously, PIPs have also been shown to regulate other channels such as the InsP3 receptor Ca^{2+} channel [122], an Na^+-activated cation channel [123], and the epithelial sodium channel (ENaC) [124]. These examples further illustrate how bilayer composition and lipid metabolites play a critical role in regulating channel activity.

Sphingolipids are of particular interest in that they are structurally important components in the lipid bilayer, playing a major role in the stability and formation of lipid rafts (discussed in the following section), as well as acting as first and second messengers in various signaling pathways [98]. Sphingomyelin signaling molecules include ceramide, sphingosine, and sphingosine-1-phosphate and have also been shown to interact with ion channels and affect their function. For example, ceramide apparently is the second messenger that mediates enhanced neuronal excitability in responses to elevated levels of pro-inflammatory nerve growth factor [125]. Ceramides have also been shown to be important inhibitors of L-type calcium channels [126] and $K_v1.3$ potassium channels [127]. With respect to connexon function, ceramides are potent inhibitors of gap junctional intercellular communication, and studies suggest that chronic release of some ceramides may act as tumor promoters [128]. Given that ceramides preferentially reside

and function in lipid rafts (see following section) and selectively displace cholesterol from these domains [129], it remains to be determined the mechanism by which ceramide (or cholesterol depletion) affects connexon function. Thus, lipid metabolites may also affect ion channel activity, and their role in the modulation of gap junctional communication has been widely observed. However, the specific molecular mechanisms of these modulatory agents remain poorly characterized.

3.3. Connexons, lipid rafts, and caveolae

As described above, cellular membranes are generally considered to exist as a fluid, liquid crystalline phase (L_α) due to the low phase transition temperature of typically polyunsaturated biological phospholipids. However, the lateral organization of the bilayer is heterogeneous, with selective association of lipids and proteins into discrete microdomains of the membrane [33]. These domains were initially defined operationally as detergent resistant membranes (DRMs) since they remained insoluble in nonionic detergents at 4 °C and had a low buoyant density. These DRMS were found to be relatively abundant in cholesterol, sphingomyelin, and glycolipids such as GM1 ganglioside [130,131]. Cholesterol packs favorably with saturated acyl chains and therefore tends to aggregate with saturated fatty acids [132] and may form "condensed" complexes that promote phase separation within the bilayer [133,134]. The insolubility of these raft domains in cold non-ionic detergent is thought to be a consequence of the higher affinity of the lipid–lipid interactions than those of lipid–detergent. Sphingolipids are distinguished by their long, largely saturated acyl chains that may pack more tightly than most glycerophospholipids that are rich in kinked unsaturated acyl chains. The formation of H-bonds with cholesterol may further stabilize the formation of microdomains enriched in sphingolipids and cholesterol [135,136]. Microdomains may be enriched in different subsets of sphingolipids differing in both in their acyl chain length and in their hydrophilic headgroup [137]. While it remains controversial whether the size and composition of these DRMs reflect raft size and composition in living cells [138], fluorescence microscopy has verified the existence of raft domains in vivo, some of which may be very small, dynamic heterogeneities [139].

The selective recruitment or exclusion of a given membrane protein from a lipid microdomain is thought to be a function of its affinity for specific lipid constituents in a given domain or by preference for an environment of a given (dis)order. For example, proteins modified with saturated acyl chain anchors such as glycosylphosphatidylinositol (GPI), myristate, or palmitate generally associate preferentially with lipid rafts [50]. These microdomains may act in signal transduction, membrane protein targeting, and clustering by sequestering or excluding distinct proteins [140]. Rafts have been shown to concentrate some GTP-binding proteins (G proteins) [141] and Src-like tyrosine kinases that are modified with long-chain acyl groups [142]. These raft microdomains move laterally and may cluster into larger patches and have been proposed to function as molecular platforms for the assembly of signaling complexes. For example, well-characterized regulatory roles of lipid rafts have been defined for the "immunological synapse" at the interface between the apposed membranes of both B and T cells and antigen-presenting cells [143–147]. In this cellular interface, lipid rafts selectively cluster essential components required for the effective initiation of immune activation (and conversely, to exclude components that would inhibit this activation). Beyond clustering effects, the structure and function of many ion channels may be modulated by the composition of the "signaling platform" in which they reside. These large supramolecular assemblies of protein networks are not uncommon since they provide the requisite colocalization needed for efficient signaling and fine regulatory control of biological processes. For example, using proteomic methods, ~75 proteins that comprise the ~2000 kDa NMDA receptor–PSD-95 complexes have been identified in simple pull-down studies [148,149]. Many of these proteins had previously been identified or implicated as having a modulatory role on NMDA receptor activity.

Functional roles for raft domains were first noted in caveolae, the specialized invaginations at or near the plasma membrane that act in endocytosis [150]. Caveolae microdomains are abundant in free cholesterol and the cholesterol-binding caveolin proteins, but lack clathrin. These specialized domains function as platforms for membrane trafficking, lipid transport, and signal transduction [151]. Several connexin isoforms (Cx43, Cx32, Cx36, and Cx46) have been shown to preferentially associate with caveolin-containing rafts and co-immunoprecipitate with caveolin-1 [146], while others are excluded (Cx26 and Cx50).

Studies of K^+ channel illustrate the differential roles of lipid rafts in channel function. Different families of K_v channels are targeted to distinct lipid raft populations [152]. For example, $K_v1.5$ channels are specifically targeted to caveolae [153], while $K_v2.1$ targets to non-caveolae rafts, and $K_v4.2$ are not raft-associated [154]. Both $K_v1.5$ and $K_v2.1$ channels are affected by lipid composition; if the cholesterol in the expressing cell is depleted with cyclodextrin, the steady-state inactivation kinetics shifts in the hyperpolarizing direction. It was not determined if this effect was due to the direct interaction of cholesterol or lipids on channel activity or by indirect signaling mechanisms due to differential activities of kinases or phosphatases. Nevertheless, the alteration of the lipid composition was observed to dramatically affect membrane excitability. For example, K_v channels are modulated by raft-segregated Src kinases [155,156].

It appears that connexins may be similarly regulated and modulated by co-localizing proteins. Most gap junction proteins have been shown to be functionally modulated by post-translational phosphorylation [30,157]. As described above, some connexins are enriched in caveolin-1-containing lipid rafts. In lens epithelial cells, Cx43 in caveolae were regulated by protein kinase Cγ recruited to this domain [147]. A molecular basis for the down-regulation of connexon activity by phosphorylation was shown in studies where the phosphorylation of S368 of Cx43 caused a conformational change that decreased connexon permeability [158]. Therefore kinases, phosphatases, and other proteins that interact with connexins have the potential to modulate junctional structure and function as a consequence of their colocalization (driven by the composition of the bilayer). For example, c-*src* is known to localize in lipid rafts, and this kinase phosphorylates Cx43 and regulates cell–cell communication [159]. Similarly, calmodulin colocalizes with and directly gates gap junction channels [160,161], providing a Ca^{2+} dependent activity. These are just two of the known examples of the modulatory effects of protein–protein interactions on connexon function that are mediated by lipid microdomains. Given the tremendous diversity of other cellular proteins that modulate gap junction composition, localization, density, and function [23], the role of lipid rafts on connexins may be profound. Rapidly developing proteomic methodologies have the potential to further elucidate the protein partners associated in gap junction plaques beyond our current knowledge [23,25,28–30]. As these proteins are defined and characterized, the role of membrane lipids and the sequestration of proteins in targeted microdomain signaling platforms will be similarly advanced.

4. Conclusions and perspective

While much of our knowledge of connexon modulation by its lipidic microenvironment to date is phenomenological, current studies indicate that these channels, similar to other membrane channels, are affected directly and indirectly by the composition of the bilayer in which they reside. Since the composition of a given bilayer is dynamic, the modulation of connexon activity by altered lipid composition affords the cell fine control over these physiologically important activities. Future studies will further refine our understanding of the roles of specific lipids and altered physicochemical parameters of the membrane on connexon function. In addition, these studies may also define the correlative changes in connexin subunit structure that underlie these functional modulations. While various laboratories have produced functional data that show the important influences of lipid composition on junctional activity, we have only recently begun to understand the structural components and molecular mechanisms involved in the interactions of lipids with connexins.

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